Differential gene expression during the initiation of oil palm (*Elaeis guineensis* Jacq.) somatic embryo development

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Introduction *In vitro* somatic embryogenesis (SE) is the only viable approach to multiply elite genotypes of oil palm, a tropical perennial monocot species that is the leading source of vegetable oil worldwide. The molecular basis of SE even in model plant species is not well understood, and molecular studies with individual crop species need to be developed for comparison. To begin to determine the molecular basis of SE in oil palm, we have initiated complementary approaches including the construction of suppression subtractive hybridisation libraries corresponding to key developmental stages, the development of an oil palm EST database, and macroarray expression analysis. In the present study we have selected from our database 227 ESTs that encode putative regulatory related products (e.g. transcription and chromatin remodelling factors and other proteins related to gene regulation, hormone metabolism and development) and examined their corresponding transcript abundance during the initiation of somatic embryo development. We have selected and validated the expression of a subset of these genes by RT-PCR and their possible significance in somatic embryo development will be discussed.

Somatic Embryo Development is Triggered by the Depletion of 2,4-D

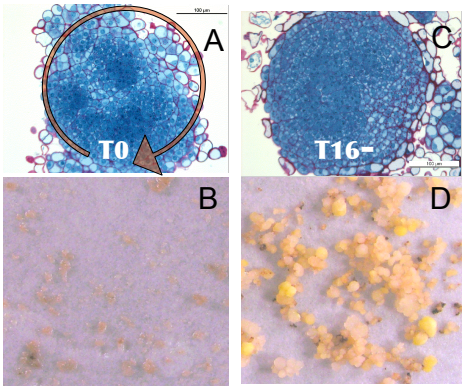

Fig. 1. The initiation of somatic embryo development. Oil palm embryogenic cells are maintained in liquid culture for 30 days (A, T0 cells) in a developmentally arrested state in the presence of 2,4-D (2,4-Dichlorophenoxyacetic acid) and remain undifferentiated when plated on solid medium for 24 days without 2,4-D (B). Cells grown for 16 days in liquid culture without 2,4-D (C, T16- cells) show no noticeable morphological differences from T0 cells, however, at the end of the 30 day cycle when plated on solid medium for 24 days without 2,4-D, embryos develop (D).

Free IAA Content Decreases During the Initiation of Embryo Development

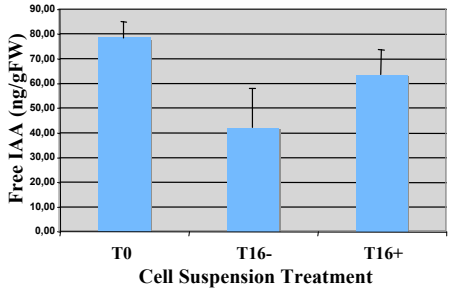

Fig. 2. Gas chromatography-mass spectrometry analysis of free indoleacetic acid (IAA) content in cell suspensions. Endogenous free IAA amounts decrease in cells grown in liquid culture without 2,4-D for 16 days (T16- cells) while remain unchanged in cells grown in the presence of 2,4-D for 16 days (T16+ cells).

Differential Gene Expression During the Initiation of Embryo Development

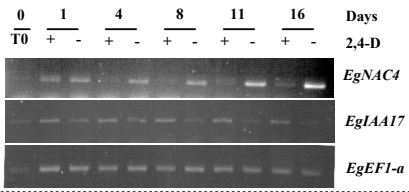

Fig. 3. RT-PCR expression analysis of genes during the first 16 days of the initiation of somatic embryo development. Previous results showed that the *EgNAC4* putative transcription factor gene increased during the initiation of embryo development, while the transcript for *EgIAA17* encoding an AUX/IAA transcriptional regulator similar to IAA17 from *Arabidopsis thaliana*, decreased during the first 16 days after removal of 2,4-D.

Developmental Regulator Macroarray Approach

Oil Palm EST Database
(sequences available: 5149)

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BLAST/Key Word Search
Regulatory Related ESTs
(e.g. transcription factors, hormones, chromatin remodeling etc… 227 total)

↓

EST cDNA Amplification by PCR

↓

PCR Product Spotted on Nylon Membranes

↓

Macroarray Hybridization
(Hybridization with labeled cDNAs)

↓

Data Analysis

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RT-PCR expression analysis
validation of selected candidates

Fig. 4. Macroarray construction strategy. To identify regulatory genes differentially expressed during the initiation of embryo development, 227 ESTs were selected from the oil palm EST database which contained 5149 ESTs from cDNA libraries constructed from different key developmental stages of somatic and zygotic embryogenesis, shoot apices and flowers. For the hybridizations, two biological repetitions were done with cDNAs made from T0, T16- and T16+ total RNA. Images were analyzed using ArrayVision software and the significance of differential expression was evaluated using the analysis of variance (ANOVA) test.

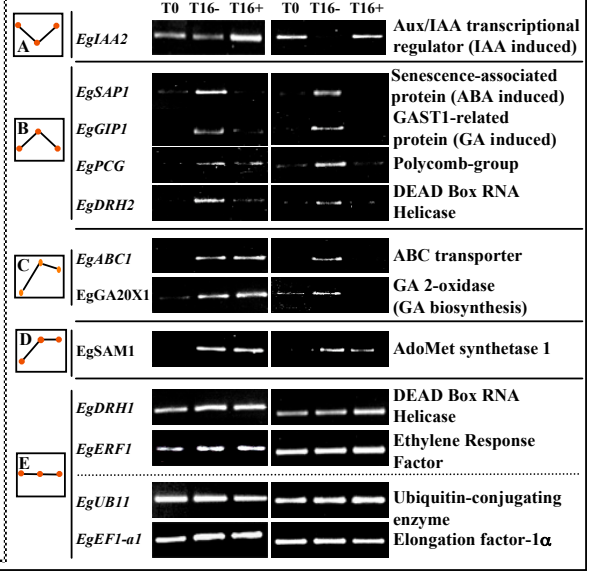
Macroarray Analysis Results: Functional Groups of Genes Expressed During the Initiation of Somatic Embryo Development

Function	Increase	Decrease	No change	Totals
Gene expression and RNA metabolism	8	2	38	48
ubiquitin/proteasome pathway	2	0	9	11
Chromatin and DNA metabolism	2	0	10	12
SAM methylation pathway	3	0	3	6
protein synthesis and processing	4	0	4	8
GA response	3	0	1	4
cell division	1	0	1	2
retrotransposon	1	0	1	2
ABA response	1	0	0	1
tumor related	0	0	1	1
cytoskeleton	1	0	1	2
embryo related	0	0	2	2
Membrane transport	1	0	0	1
programmed cell death	0	0	1	1
unknown abundant cluster	0	0	1	1
auxin response	0	1	2	3
ethylene response	0	0	2	2
nuclear import	0	0	2	2
Oil palm mantled marker	0	1	1	2
Totals	**27**	**4**	**81**	**112**

Table 1. Functional categories of ESTs corresponding to genes with reproducible expression profiles in T0 and T16- cells. The identification of genes differentially expressed after transferring suspension cells (T0) into medium without 2,4-D for 16 days (T16- cells) may provide candidates involved in the initiation mechanisms of somatic embryo development. A total of 27 transcripts increased in T16- cells ($P < 0.05$ and ratio >2) , while only 4 transcripts decreased ($P < 0.05$ and ratio < 0.5). The majority (112) of the transcripts had similar abundance in T0 and T16- cells. Several of the genes differentially expressed were related to hormone response or metabolism including auxin, gibberellin (GA) and abscisic acid (ABA). From the 227 ESTs initially selected,, 115 corresponded to transcripts with inconsistent abundance between the two biological repetitions and were eliminated from further analysis. Only the results for genes with expression patterns that were confirmed in both repetitions are presented.

Fig. 5. RT-PCR expression analysis of selected candidates. Validation of macroarray data was based on cDNAs isolated from two biological repetitions (RT-PCR1 and 2). The transcript profiles of 11 genes selected for validation, in addition to *EgEF1-α1* as a housekeeping control, could be divided into five groups based on their transcript accumulation in T0, T16- and T16+ cell cultures. In profile **A**, *EgIAA2* accumulated in T0 and T16+ cells in both replications. In contrast, the *EgIAA2* was either undetected or less abundant in T16- cells. In profile **B**, a total of six transcripts including *EgSAP1*, *EgGIP1*, *EgPCG*, *EgDRH2*, *EgABC1* and *EgGA2OX1*, accumulated preferentially in T16- cells in both repetitions. Four of those genes, *EgSAP1*, *EgGIP1*, *EgPCG* and *EgDRH2*, had higher expression in T16- than in T0 and T16+ cells in both RT-PCR1 and 2. In contrast, transcript amounts for *EgABC1* and *EgGA20X1* were variable in the two biological repetitions in T16+ cells (profile **C**). The *EgSAM1* transcript was unique (profile **D**), with similar accumulation in T16- and T16+ cells and low or undetectable in T0 cells. Finally, profile **E** included constitutive accumulation of transcripts *EgDRH1*, *EgERF1*, *EgUB11* and *EgEF1-α1* in T0, T16- and T16+ cells. The transcript abundance of *EgUB11* and *EgEF1-α1* was as expected, similar in all the treatments, whereas expression profiles of the genes *EgDRH1 and EgERF1* were different from those observed in the macroarray analysis. RT-PCR analysis 1 validated the macroarray transcript profiles for 9 of the 11 genes examined with *EgDRH1* and *EgERF1* the exceptions. Both the macroarray and RT-PCR results indicate that the expression profiles for T0 and T16- cells are less variable than with T16+ cells.

RT-PCR Analysis Validation of Macroarray Analysis Confirms Differential Expression



Conclusions and Perspectives Physiological (decrease in free IAA) and molecular (differential transcript accumulation) changes occur by 16 days in cells initiated to develop somatic embryos. 27 of the genes examined revealed an increase in transcript abundance while only 4 transcripts decreased in abundance at 16 days after 2,4-D removal (T16-). Several of the genes found to be differentially expressed indicate changes in auxin, gibberellin, and ABA response or metabolism are occurring in cells initiated to develop embryos. The combination of macroarray and RT-PCR analysis enables the selection of candidate genes differentially expressed during the initiation of embryo development for functional studies related to changes in hormone physiology and coordinated gene expression.